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Effects of operating parameters on hydrogen production from raw wet steam-exploded cornstalk and two-stage fermentation potential for biohythane production

Zhidan Liu[a,1], Qian Li[c,1], Chong Zhang[b], Linjun Wang[b], Bing Han[d], Baoming Li[a], Yuanhui Zhang[e], Hongzhang Chen[f], Xin-Hui Xing[b,*]

[a] Laboratory of Environment-Enhancing Energy (E2E), College of Water Resources and Civil Engineering, China Agricultural University, Beijing 100083, China
[b] Department of Chemical Engineering, Tsinghua University, Beijing 100084, China
[c] Department of Environmental Engineering, University of Science and Technology Beijing, Beijing 100083, China
[d] China's National Institute for Clean-and-Low-Carbon Energy (NICE), Beijing 102209, China
[e] Department of Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[f] National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China

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ABSTRACT

Biohythane (biohydrogen + biomethane) production from agricultural residue is a win–win solution for the supply of renewable energy and valorization of waste biomass. This study reported the first investigation on hydrogen fermentation directly using raw wet steam-exploded cornstalk (SC) without any further processing for drying or detoxification. The effects of key operating parameters (feedstock concentration, initial pH and heat treatment of seed sludge) were systematically studied. The suitable conditions for hydrogen fermentation from the wet SC were the feedstock concentration at 200 g L^{-1} (TS, 6–8%), pH at 6.5 and seed sludge without heat treatment. In addition, compared to one-stage biomethane fermentation, the two-stage biohythane fermentation by integrating hydrogen fermentation with biomethane production from SC led to the hydrogen and methane yields of 12 and 195 L kg^{-1} TS^{-1}, respectively, corresponding to an increased energy recovery of 26%, reduced fermentation time and facilitated conversion of volatile fatty acids. These results demonstrated the feasible energy-efficient biohydrogen or biohythane production from the wet steam-exploded cornstalk, implying the promising potential of this method for harvesting clean hythane vehicle fuel from agricultural biomass.

1. Introduction

The energy crisis is becoming a global issue. Hydrogen, as a clean and efficient renewable energy, is considered to be the best alternative to fossil fuels [1–4]. However, commercialization of hydrogen energy is hampered by a cost-intensive process. Hythane, a mixture of hydrogen and methane, has attracted significant attention as a transit form of pure hydrogen in the near term [5]. With the addition of hydrogen to methane, hythane has been noted to exhibit obvious advantages over compressed natural gas as a vehicle fuel, such as extended flammability range, shortened quenching distance, reduced greenhouse gas emissions, and improved fuel efficiency [6]. Hythane could be sustainably produced from biomass through microbial fermentation (biohythane) [7,8]. With the development of agriculture, the yield of agricultural residues has increased up to about 700 million tons a year in China [9]. Therefore, utilization of agricultural residues for the production of biohythane through two-stage anaerobic fermentation is an important way to valorize the agricultural waste, reduce environmental pollution, and, to some extent, complement the constrained energy supply.

Lignocellulosic agricultural biomass, such as cornstalk, has natural recalcitrance with a highly rigid three-dimensional structure mainly consisting of cellulose, hemicellulose, and lignin, making it very difficult to degrade and transform [10]. Steam explosion has already been recognized as an efficient approach to breaking up the structure of lignocelluloses, where the changes in the water forms serve as the main factor [11,12]. However, the hydrolysate obtained after steam explosion contains many

* Corresponding author. Tel.: +86 10 6279 4771; fax: +86 10 6278 7472.
E-mail address: xhxing@tsinghua.edu.cn (X.-H. Xing).
[1] These authors contributed equally to this work.

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fermentation inhibitors, including phenolic compounds, furfural and acetic acid [13]. An additional step of detoxification is generally needed to remove these inhibitors, including the use of milk of lime [14], organic solvent extraction [15], activated carbon adsorption [16], concentration under vacuum [17], and ion exchange [18]. Moreover, steam-exploded cornstalk (SC) always needs to be dried before the use in the subsequent bioprocess, thus making the whole process energy-intensive, water-unsustainable, and industrially undesirable. It is therefore of crucial importance to study the possibility of directly utilizing wet SC in anaerobic fermentation. However, so far there is no report on the effects of the wet steam-exploded cornstalk on anaerobic fermentation for the production of either biohydrogen, biomethane or biohythane.

The purposes of the current study were to (1) investigate the feasibility of hydrogen fermentation directly using wet SC without detoxification and drying; (2) evaluate the influence of process parameters (feedstock concentration, initial pH and heat treatment of seed sludge) on hydrogen fermentation by using a normal-pressure batch bioreactor; and (3) examine the biohythane production potential from wet SC by using two-stage anaerobic fermentation.

2. Materials and methods

2.1. Seed sludge, substrate and medium

The anaerobic sludge, sampled from an anaerobic digester of Xiaohongmen Wastewater Treatment Plant (Beijing, China), was used as the seed sludge. Three different SC were used as substrates: a dried one (TS, >90%; VS/TS, 84–86%) taken from a factory in Shandong (SDSC); a wet one (TS, 28–30%; VS/TS, 76–80%) directly taken from Laihe Company (LHSC). The concentrations of HMF and FUR in LHSC were in ranges of 90–150 and 60–165 mg L^{-1}, respectively; and another dried one (TS, >90%; VS/TS, 90–96%) taken from Prof. Chen Hongzhang's Laboratory (Institute of Process Engineering, Chinese Academy of Sciences) (LASC). The medium contained the following (L^{-1}): yeast extract, 2.0 g; $(NH_4)_2SO_4$, 1.3 g; KH_2PO_4, 1.5 g; $K_2HPO_4{\cdot}3H_2O$, 2.9 g; $CaCl_2$, 0.075 g; $MgCl_2{\cdot}6H_2O$, 0.2 g; and $FeSO_4{\cdot}7H_2O$, 1.25 mg. The pH of all the substrates was 6–6.5 unless otherwise stated.

2.2. Experimental system and procedure

The experiment device was a normal-pressure bioreactor system, consisting of 250-ml glass flask with a working volume of 150 ml, gas-tight plastic tubes, sampling valve, and gas balloon. The glass flask served as the anaerobic vessel for fermentation, the produced gas was measured using the gas sampling valve and collected by gas balloon, and the fermented broth was sampled by the sampling port embedded in the flask. The feasibility study of hydrogen fermentation from the wet SC was first investigated by examining the effects of key operating parameters, including SC types and concentration, initial pH and heat pretreatment of seed sludge. The flask bioreactors containing substrate were degassed with pure nitrogen for 30 min to reach anaerobic conditions prior to use. To determine the influence of substrate concentration, 40, 100, 200, 400, and 800 g L^{-1} of substrate were tested, respectively. To evaluate the effect of pH on anaerobic fermentation, the medium with initial pH of 5.5, 6.5, and 7.5 was prepared. With regard to detection of the effect of heat pretreatment of seed sludge, the experiments were performed using a water bath with temperatures controlled at 50, 80, and 100 °C, respectively. 7% (w/v) of the boiled anaerobic sludge (70 g L^{-1}) was inoculated in a flask culture for biohydrogen production [19].

The biochemical potential for coproduction of hydrogen and methane (biohythane) [7] from SC was then investigated under the optimized conditions compared to one-stage biomethane process. The one-stage biomethane experiment was carried out using an initial pH of 7.5, whereas the two-stage fermentation for biohythane production was performed using an initial pH of 6.5 for hydrogen fermentation, followed by adjusting pH to 7.5 for the subsequent methane fermentation when hydrogen production was ceased in the same flask culture system.

2.3. Analytical methods

Gas samples were analyzed by using a gas chromatograph equipped with a thermal conductivity detector (TCD) and a column packed with TDX-01 (GC112A, China) [20]. The detected gases included hydrogen, oxygen, and methane. Metabolic intermediates during microbial fermentation were analyzed by a high performance liquid chromatography (HPLC; Shimadzu 10A) equipped with a refractive index detector (RID) and a Shodex RSpak KC-811 column. $HClO_4$ (1 g L^{-1}) was used as the mobile phase at a flow rate of 1 ml min^{-1}. The samples were centrifuged (12,000 rpm, 10 min) and the supernatant was filtered using a 0.22-mm membrane filter before use. The detected intermediates included volatile fatty acids (VFAs) and ethanol.

The concentrations of soluble sugars were measured by employing the phenol–sulfuric acid method [21]. Cellulose, hemicelluloses, and lignin in the pretreated and fermented cornstalks were evaluated according to the procedures reported by National Renewable Energy Laboratory (NREL) [22].

The utilized glucose equivalent was calculated based on carbon balance during microbial fermentation, and its detailed description has been given elsewhere [19]. Energy recovery was calculated by dividing the combustion values of hydrogen and methane produced by that of cornstalk [19]. The combustion values of hydrogen, methane, and glucose are 280, 864, and 2870 kJ mol^{-1}, respectively, and a detailed description has been given elsewhere [19].

3. Results and discussion

3.1. Hydrogen fermentation using SC

3.1.1. Effects of SC types and concentrations on hydrogen fermentation

Table 1 shows the comparison of hydrogen productivity using different SC as feedstocks. LHSC achieved a maximum hydrogen yield of 10.21 L kg^{-1} TS^{-1}. Compared with the dried SC, such as SDSC and LASC, LHSC contained more soluble sugar and VFAs, which contributed to more hydrogen production.

The effect of feedstock concentrations on hydrogen fermentation was then studied using LHSC. With the increase in the substrate concentration from 40 to 200 g L^{-1}, the hydrogen yield increased up to 10.41 L kg^{-1} TS^{-1}. However, the hydrogen production ceased when the substrate concentration was 400 g L^{-1} and 800 g L^{-1}. A similar phenomenon was observed through the analysis of metabolites after fermentation. When the substrate concentration was increased up to 200 g L^{-1}, acetic acid reached its maximum (5.54 mM), whereas decreased VFAs were observed when the substrate concentration reached 400 g L^{-1}. Substrate concentration is an important factor for anaerobic fermentation. The current results demonstrated that 200 g L^{-1} was a suitable concentration for hydrogen fermentation. The substrate at high concentration (400 or 800 g L^{-1}) might result in uneven mass transfer and contain a high amount of fermentation inhibitors, thereby suppressing hydrogen fermentation according to the study by Li and Chen [13].

Table 1
Comparison of hydrogen productivity with different SC ($n = 3$). SDSC, dried SC from a factory in Shandong; LASC, dried SC from Prof. Chen's Laboratory (IPE); LHSC, wet SC directly taken from Laihe Company.

Feedstock	Concentration ($g\,L^{-1}$)	TS (%)	Volumic H_2 production ($L\,L^{-1}$)	Gas productivity ($L\,kg^{-1}\,TS^{-1}$)	H_2 productivity ($L\,kg^{-1}\,TS^{-1}$)	Final pH
SDSC	20	1.8	0.001 ± 0.001	12.04 ± 5.37	0.072 ± 0.061	6.12
LASC	20	1.9	0.02 ± 0.007	28.95 ± 3.68	1.01 ± 0.35	5.58 ± 0.12
LHSC	100	4	0.28 ± 0.001	41.23	10.21 ± 0.03	5.15 ± 0.01

3.1.2. Effect of initial pH on hydrogen fermentation

At an initial pH of 6.5, the hydrogen yield exhibited significant changes from 0 to 86 h (Fig. 1A). At 86 h, the hydrogen yield approximately reached the maximum value of 3.76 $L\,kg^{-1}\,TS^{-1}$. The hydrogen yield did not change much after 86 h. Unlike the value at pH 6.5, the maximum hydrogen yields at pH 7.5 and 5.5 were only 1.37 and 0.14 $L\,kg^{-1}\,TS^{-1}$, respectively. The hydrogen content reached its maximum of 26% at pH 6.5 and 37 h. Obviously, the initial pH of 6.5 was suitable for hydrogen fermentation, because hydrogen fermentation is generally carried out under acidic conditions, especially when anaerobic sludge is used as inoculum. Acidic condition may suppress methane production. Zhang et al. [23] reported that NADH generated in the EMP pathway also regulated hydrogen production through the balance of NAD^+/NADH under acidic conditions. A higher initial pH of more than 7.0 may stimulate biomethane process, thus decreasing hydrogen productivity. The optimal pH was suggested in a wide range of 4.0–7.0 depending on many factors, such as inoculums and substrates [3]. Moreover, analysis of the fermented liquid products (Fig. 1B) revealed that lactic acid was induced at pH 5.5, whereas more acetic acid and propionic acid were produced at pH 7.5. These results implied that control of pH is of importance to hydrogen fermentation from the wet steam-explored in terms of both hydrogen productivity and VFA composition, since more acetic acid would be desirable for the subsequent methane fermentation.

3.1.3. Effects of seed sludge heat treatment on hydrogen fermentation

Fig. 2 shows the effect of heat treatment (0, 50, 80, and 100 °C) of seed sludge on hydrogen fermentation. Interestingly, the hydrogen yields using the seed sludge without treatment and after heat treatment at 100 °C were similar, at around 9 $L\,kg^{-1}\,TS^{-1}$, higher than those achieved at 50 and 80 °C. The analysis of metabolites after fermentation (Fig. 2B) showed that higher heating temperature resulted in increased production of propionic acid and formic acid, compared with the control. Heat treatment at 100 °C is generally regarded as an efficient method to inactivate the hydrogenotrophic bacteria and enrich hydrogen-producing spore-forming bacteria, such as *Clostridium* sp. [3,24]. In comparison, these results revealed that heat treatment of seed sludge had little influence on hydrogen fermentation from the wet SC. A possible reason was the complexity of the wet SC containing fermentation inhibitors, which might selectively favor hydrogen fermentation according to Li and Chen [11]. We recently found that fermentation inhibitors, specifically

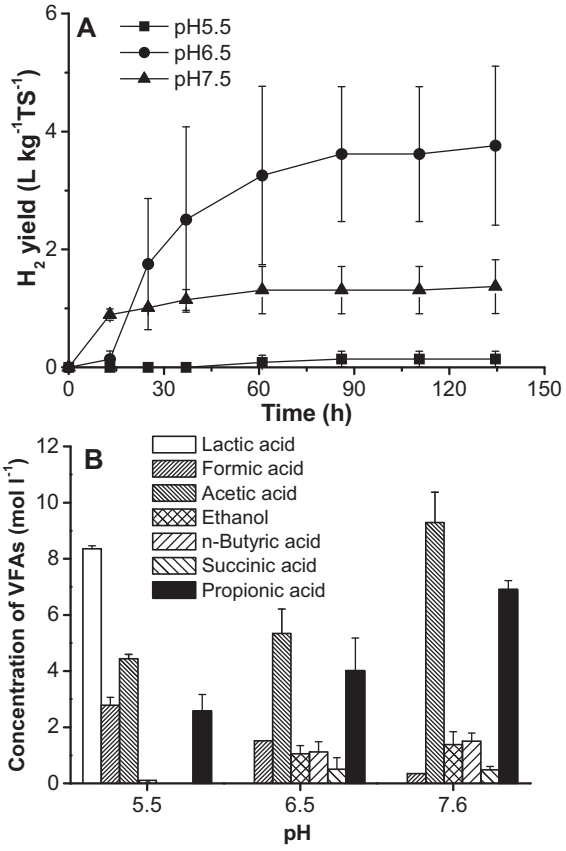

Fig. 1. Effect of initial pH on hydrogen fermentation from LHSC ($n = 3$). (A) Hydrogen yields; (B) analysis of metabolites (mM) after fermentation.

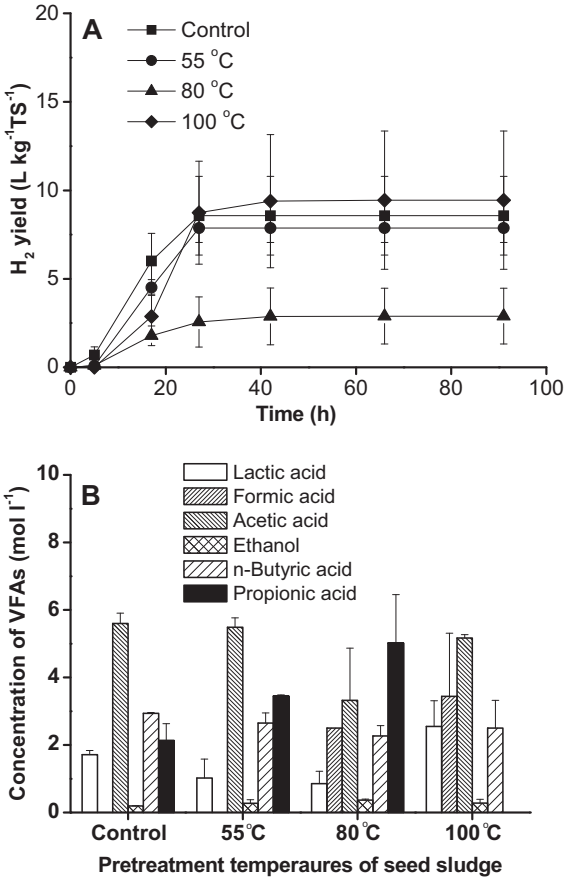

Fig. 2. Effect of pretreatment temperatures for the seed sludge on hydrogen fermentation from LHSC ($n = 3$). (A) Hydrogen yields; (B) analysis of metabolites (mM) after fermentation.

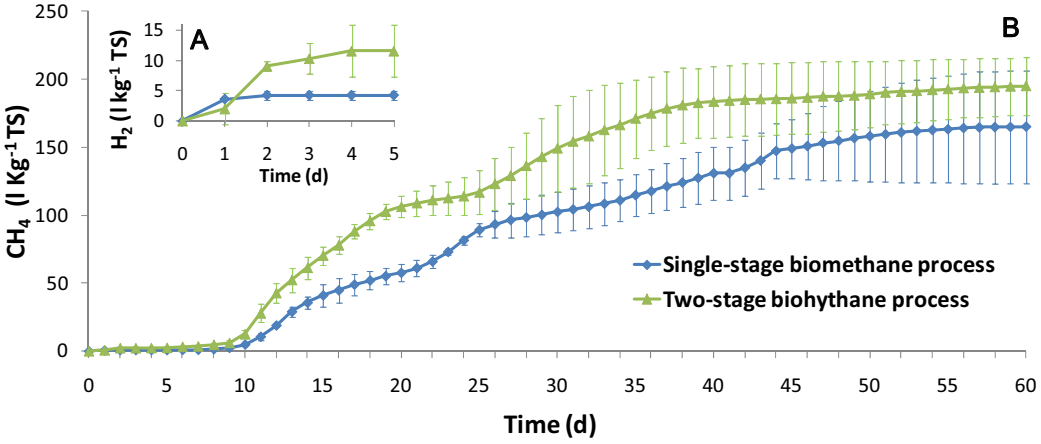

Fig. 3. Comparison of gas biofuels production from wet LHSC through one-stage biomethane process and two-stage biohythane process ($n = 3$). (A) Hydrogen production; (B) methane production.

furfural and 5-hydroxymethyl furfural, were degraded through hydrogen fermentation and even enhanced the biohydrogen production [25]. However, further study on the mechanism needs to be carried out.

3.2. Potential for biohythane production from wet SC by two-stage hydrogen and methane fermentation

Based on the optimized conditions for hydrogen fermentation directly from the raw wet SC, the potential for coproduction of hydrogen and methane (biohythane) via hydrogen fermentation followed by methane fermentation was evaluated by using a self-developed normal-pressure bioreactor system. Anaerobic sludge of 70 g L^{-1} (7%, w/v) was used as the inoculum and 200 g L^{-1} of LHSC was used as the feedstock. Compared with the one-stage methane process, the two-stage biohythane process significantly enhanced hydrogen production by 49% and methane production by 25% (Fig. 3). This corresponded to a 26% increase in the total energy recovery. Under the conditions for biohythane production, a hydrogen yield of 12 L kg^{-1} TS^{-1} and a methane yield of 195 L kg^{-1} TS^{-1} were achieved, corresponding to an energy recovery of more than 100%. This might be due to the fact that the coproduction of biohydrogen and biomethane from biomass is an endothermic reaction, with a theoretical energy recovery of more than 100% [7]. In addition, as shown in Fig. 3, to obtain the same energy recovery, the required time for the two-stage biohythane process was much shorter than that for the single-stage methane process. Analysis of fermentation metabolites (Table 2) showed that all the VFAs were converted into gas biofuels after 60 days' operation. One interesting finding was that one-stage biomethane fermentation generated much higher propionic acid (11–12 mM) at 22 and 30 days than the two-stage biohythane process, suggesting that more efficient conversion of propionic acid into acetic acid was achieved in the biohythane process. Chu et al. [26] reported similar results using food waste as the substrate for the coproduction of biohydrogen and biomethane. Compared to easily biodegradable food waste, cornstalk has the cellulosic recalcitrance with a highly rigid three-dimensional structure, making it very difficult to degrade and convert. Cornstalk was hydrolyzed and liquefied through microorganism [19] or alkali chemicals [27] before its utilization for the coproduction of biohydrogen and biomethane. However, the direct conversion of wet SC containing liquid and solid organics into biohythane has not been reported elsewhere. Table 3 indicated that hydrolysis of cornstalk was not significantly affected by the

Table 2
Analysis of fermentation metabolites (mM) during biochemical potential study of LHSC ($n = 3$).

Time (days)	Process	Lactic acid	Formic acid	Acetic acid	Propionic acid	*n*-Butyric acid	Valeric acid
22	O	0	0	0	0	0	0
	T	0	1.14 ± 0.45	1.95 ± 1.13	0.20 ± 0.28	4.27 ± 0.64	0
30	O	0.71 ± 1.00	0	1.27 ± 0.90	12.95 ± 0.33	0	0
	T	0	0	1.39 ± 0.55	6.19 ± 7.83	0.18 ± 0.25	0.30 ± 0.42
38	O	0	0	0.97 ± 0.18	11.56 ± 1.26	0	0
	T	0	0	1.45 ± 0.07	0	0	0
45	O	0	0	1.35 ± 0.51	3.03 ± 1.33	0	0
	T	0	0	0.89 ± 0.27	0.20 ± 0.28	0	0.16 ± 0.23
60	O	0	0	0	0	0	0
	T	0	0	0	0	0	0

O, one-stage biomethane process (initial pH 7.5); T, two-stage biohythane process (initial pH 6.5 and pH was adjusted to 7.5 after the hydrogen production).

Table 3
Analysis of biomass components after anaerobic fermentation of LHSC.

Process	Cellulose (g g^{-1})	Hemicellulose (g g^{-1})	Lignin (g g^{-1})	Ash (g g^{-1})	Hydrolysis rate of SE cornstalk (%)	Hydrolysis rate of cornstalk (%)
O	n.d.	n.d.	0.25	0.37	45.09	56
T	n.d.	n.d.	0.18	0.32	47.97	58

O, one-stage biomethane process (initial pH 7.5); T, two-stage biohythane process (initial pH 6.5 and pH was adjusted to 7.5 after the hydrogen production); n.d., not detectable.

fermentation stages. Cellulose and hemicellulose were completely degraded and the total hydrolysis rates were around 50–60% after fermentation under both conditions.

The direct use of the raw wet SC for biohydrogen and biohythane production as shown in this study was a useful approach for biorefinery of agricultural biomass. Most studies using SC for fermentation had to undergo energy-intensive drying or extra detoxification before fermentation [13]. Compared with the fermentation using dried or detoxified SC (TS > 90%) as substrate, almost 60% of water could be saved by directly using wet steam-exploded cornstalk (TS, 28–30%), thus making the process more feasible. Furthermore, pH of the wet SC was 6–6.5, which is particularly suitable for hydrogen fermentation, thus saving the operating cost for pH adjustment. However, the sustainability of the biohythane process needs to be considered. This study is focused on the potential of biohythane generation from wet SC. Further work is needed to investigate the biohythane production in continuous operation and evaluate its process economy, which is ongoing now.

4. Conclusions

The current study demonstrated that raw wet SC could be directly used for hydrogen fermentation without any further step for detoxification or drying. The analysis of gas production and metabolites indicated that initial pH and feedstock concentration greatly impacted hydrogen productivity, whereas heat treatment was found unnecessary for hydrogen production from the wet SC. A maximum hydrogen yield ($10\,L\,kg^{-1}\,TS^{-1}$) and hydrogen content (36.83%) were achieved under the optimized conditions at $200\,g\,L^{-1}$ of substrate, initial pH of 6.5 and seed sludge without heat treatment. Furthermore, the two-stage biohythane process led to a maximum hydrogen yield of $12\,L\,kg^{-1}\,TS^{-1}$ and methane yield of $195\,L\,kg^{-1}\,TS^{-1}$, 26% higher in the total energy recovery than one-stage biomethane fermentation with reduced fermentation time, indicating the potential of this method for harvesting clean hythane vehicle fuel from waste biomass.

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